

PROTEIN VACCINES AGAINST POXVIRUSES

This application claims the benefit of priority from provisional application No. 60/722,082, filed Sep. 21, 2005, and the entire contents of that application are incorporated herein by reference.

Viruses in the family Poxviridae, including vaccinia virus (VACV), variola virus (smallpox), and monkeypox virus, are characterized by a large linear double-stranded DNA genome (130-300 kb) packaged in a relatively large virion (.about.350.times.270 nm), and a cytoplasmic site of replication (reviewed by Moss, 1996, In "Fields Virology", D. M. Knipe et al. Eds., vol. 3, pp 2637-2671. Lippincott-Raven, Philadelphia). Assembly of VACV virions begins with condensation of dense granular material into membrane-wrapped particles called intracellular mature virions (IMV). Recent findings indicate the IMV are wrapped by a single membrane (Hollingshead et al., 1999, *J. Virol.* 73, 1503-1517) rather than a double membrane as previously reported. IMV are then enveloped in two additional membranes derived from the trans Golgi to form multiple membrane-wrapped particles called intracellular enveloped virions (IEV) (Schmelz et al., 1994, *J. Virol.* 68, 130-147). IEV are moved, possibly by actin polymerization (Cudmore et al., 1995, *Nature* 378, 636-638), to the cell periphery, where the outermost membrane fuses with the cell plasma membrane, exposing a cell-associated enveloped virion (CEV) (Blasco and Moss, 1991, *J. Virol.* 65, 5910-5920). CEV are released from the cell as extracellular enveloped virions (EEV), which play a role in long-range spread of the virus (Payne, 1980, *J. Gen. Virol.* 50, 89-100). IMV released from disrupted cells and EEV are both infectious forms of VACV.

Smallpox and monkeypox are caused by closely related orthopoxviruses, variola virus and monkeypox virus, respectively. While smallpox is highly transmissible among humans and causes death in approximately one-third of infected individuals, monkeypox is transmitted less efficiently and has a lower mortality rate (see reference citations 1, 2). Smallpox has been eradicated worldwide and it is not a public health concern except for its possible use as a bioterrorism threat. Outbreaks of monkeypox have often been reported in Africa since 1970 (3, 4) and, unexpectedly, in the U.S. (5, 6). The U.S. outbreak (thirty-seven confirmed cases within a few weeks) was due to exposure to monkeypox-infected prairie dogs that had contracted the disease from imported African rodents (5). While an embargo on importation and sale of African rodents would prevent exposure, this episode nevertheless indicates the unpredictable nature of zoonotic infection of humans. Thus, the development of safer vaccines or drugs to prevent or treat such infection will serve the public.

The current smallpox vaccine is a live VACV that has many drawbacks including: adverse reactions, scarring, ocular autoinoculation, dissemination in immunocompromised persons, and dwindling stocks. Adverse events range from the non-serious (e.g., fever, rash, headache, pain, and fatigue) to life-threatening (e.g., exzema vaccinatum, encephalitis, and progressive vaccinia). Serious adverse events that were reported during past and present smallpox vaccination programs include myocarditis and/or myopericarditis. Moreover, live VACV vaccines are problematic because lesion-associated virus at the site of vaccination is infectious and can be inadvertently spread to other parts of the body (e.g., ocular auto-inoculation) and to other individuals (i.e., contact vaccinia). Contact vaccinia has been a significant problem for family members of military vaccines.

Cell culture-derived vaccines are being developed; however, these vaccines are also live viruses and pose many of the

same drawbacks that plague the current vaccine. The existing smallpox vaccine Dryvax, a live vaccinia virus (VACV), protects against smallpox and monkeypox, but is contra-indicated in immunocompromised individuals. (7). There are numerous adverse events that can affect both the vaccinee and persons in contact with the vaccinee (e.g., contact vaccinia) (8, 9).

Monkeypox virus infection of healthy rhesus macaques appears to be a suitable model to study protective immune responses against monkeypox (10). Indeed, macaques vaccinated Dryvax are protected from monkeypox (10-14). Recent data have shown that the major mode of protection from monkeypox afforded by the current non-attenuated smallpox vaccine is mediated by antibodies (14). Depletion of either CD4⁺ T cells or CD8⁺ T cells in vaccinated animals prior to monkeypox virus challenge does not affect survival, whereas B cell depletion before and during immunization abrogates vaccine-induced protection. Accordingly, passive administration of VACV antibodies confers protection from subsequent lethal monkeypox (14).

The definition of VACV protective antigens has been limited by the complexity of the VACV proteome that encodes some two hundred proteins. Of the approximately 200 genes that comprise the vaccinia genome, only five encode proteins that are known to elicit a neutralizing antibody response including: H3L (Gordon et al., 1991), A27L (Lai et al., 1991; Rodriguez and Esteban 1987), B5R (Galmiche et al., 1999), D8L (Hsiao et al. 1999), and L1R (Ichihashi et al., 1994; Wolffe et al., 1995). Given the structural complexity of VACV, there may be other neutralizing antigens not yet identified. In addition, the A33R gene encodes a protein that elicits a non neutralizing antibody response that is, nevertheless, protective (Schmaljohn unpublished; Galmiche et al., 1999, *Virology* 254, 71-80).

Nevertheless, proteins L1R and A27L, specific to the intracellular mature virus (IMV), and proteins A33R and B5R, specific to the extracellular enveloped virus (EEV), have been shown to be immunogenic and protected mice from VACV (15-17). In addition, vaccination with a single protein, A33R, was shown to protect against a lethal challenge with ectromelia virus, which is a highly virulent natural pathogen in mice (18). EEV are produced when IMV wrap in additional cellular membranes, move to the cell surface, and release from the cell (19). Both the IMV and EEV forms of poxviruses are infectious. Recently, protection from monkeypox-induced severe disease was also observed following gene gun immunizations with only four VACV DNA plasmids encoding these four proteins (10). U.S. Pat. No. 6,562,376 describes DNA vaccines against poxviruses using IMV and EEV proteins, and this patent is incorporated herein by reference in its entirety.

In light of the problems associated with current live poxvirus vaccines, there is clearly a need for a safe, noninfectious, effective and relatively convenient vaccine alternative that confers protective immunity to poxviruses, which could be used in endemic areas to prevent the suffering caused by diseases such as smallpox.

SUMMARY OF THE INVENTION

Monkeypox transmits poorly from person to person and has a lower rate of mortality (4-15%) (1, 3) compared to smallpox (~30%). However, in contrast to smallpox, monkeypox cannot be eradicated. The virus has an unknown animal reservoir and the existence of more virulent strains is plausible. The 2003 U.S. human monkeypox outbreak (5) was the